

## Chapter 2

# Multipotency and Tissue-Specific Stem Cells

**Keywords** Tissue-specific stem cells · Liver progenitors · Skin stem cells · Gut stem cells · Skeletal muscle stem cells · Cardiovascular progenitors · Neuronal stem cells (NRCs)

## 2.1 Tissue-Specific Stem Cells from Organs Capable of Extensive Regeneration

### 2.1.1 HSCs Versus Other Tissue-Specific Stem Cells

The existence of tissue-specific stem cells makes much sense, in view of the high repair potential of organs such as the blood, skin, gut, and liver (Box 2.1). Many tissues of the adult organism are supposedly incapable of regeneration. The nervous system is one example. In general, a clear distinction has been made between tissues that undergo constant repair, and tissues that do not. It has been assumed that only the former contain stem cells devoted to the repair of the tissue in which they reside. Therefore, subsequent to the discovery of HSCs, attempts were made to identify other cells in the organism that are tissue-specific stem cells. There are manifold reasons why these studies encountered extreme difficulties; primarily, the hemopoietic system can be easily depleted by radiation to almost zero, without harming other body functions (Chapter 1). To date, such simple and specific depletion is not possible with any other tissue. Secondly, HSCs can be transplanted via the peripheral blood route, and find their way into the bone marrow compartment effectively, while most other stem cells cannot function similarly. Thirdly, HSCs can be harvested by flushing the bone marrow while creating single cell suspensions, whereas solid organs should be treated vigorously with proteolytic enzymes to produce single cells. The transplantation of such cells is done locally, since they would not travel effectively through the blood circulation. Fourthly, due to the harsh conditions needed for the isolation of cells from solid tissues, the analysis of markers of cell subpopulations is by far more complicated than the situation with hemopoietic tissue cells. Finally, and most importantly, the HSC has been examined at the

single cell level, following in vivo administration, and showed the expected ability to reproduce itself and to give rise to mature progeny in a multipotent fashion. Such experiments are either scarce or altogether absent in the analysis of other stem cell types. For these reasons, the study of tissue-specific stem cells other than HSCs is lagging.

### **Box 2.1 Regeneration versus repair**

A distinction is made herein between regenerating organs (blood and liver) versus tissues with repair capacity (gut and skin). Regeneration is defined as the formation of a tissue or organ following massive amputation or depletion. The liver will regrow after amputation of over 50% of its mass, and hemopoiesis can be restored by a single stem cell after total destruction of the tissue. Repair or repopulation is defined as the correction of diffuse damage, that has depleted the tissue or organ but has not destroyed the entire tissue structure or eliminated a major part of the organ. The gut will not re-form a new part after amputation, and removal of vast areas of skin will result in scar formation. Nevertheless, smaller-scale damage to these tissues will be followed by repair.

Cell death and renewal are constantly occurring in the liver, skin, and gut. It was therefore anticipated that these organs should contain tissue-specific stem cells. In Chapters 5 and 6, stem cells are redefined, and it is demonstrated that most of the cell types discussed within this chapter are not stem cells. However, for the sake of discussion, and in order to make it easier for the reader to identify the cells under discussion with those reported in the literature, the prevalent nomenclature will, at this point, be used.

### ***2.1.2 Liver Progenitor Cells***

The mammalian liver, much like the hemopoietic system, has a remarkable regenerative capacity. Upon amputation of over half of this organ, the remaining part will grow back to assume the shape and size of the original liver. This clearly is not the case for other organs such as the kidney. The outstanding regenerative capacity of the liver may depend upon liver-resident stem cells. However, it is astonishing to realize that relatively little is known about hepatic cell sub-populations. Mature hepatocytes are by themselves capable of proliferation, and account for part of the regenerative potential of the liver. However, this capacity is thought to be limited, and, upon major amputation or toxic tissue damage, the proliferation of mature liver cells may not suffice to repair the damage. It is therefore anticipated that the liver contains immature precursor cells that perform the regenerative process.

Hepatic precursor cells have been studied in divergent settings, including the embryonic versus adult liver, and in several models of liver injury. In the adult

liver, several candidate hepatic precursor cells have been described. Reid and colleagues studied oval cells, which are smaller than mature hepatocytes. These cells could be maintained in culture on type IV collagen and laminin, and required the addition of complex lipids, insulin-like growth factor II and GM-CSF, along with the support of stromal cells (Brill et al. 1993). Oval cells are located in the terminal bile ducts, in the canals of Hering, which are the sites of transition from the bile ductules to hepatocytes. Oval cells are supposed to express the surface antigens A6 and G7 (Engelhardt et al. 1990, Petersen et al. 2003), Thy-1, which is also found in hemopoietic cells (Petersen et al. 1998), as well as the liver proteins  $\alpha$ -fetoprotein, albumin, and cytokeratin-19. In one study, based on the shared properties of HSCs and oval cells, the latter were isolated from the liver and were shown to respond to somatostatin by increased migration (Jung et al. 2006). Glypican-3, a heparan sulfate proteoglycan, is found in hepatic progenitor cells (Grozdanov et al. 2006). Transcriptional profiling of embryonic liver cells capable of differentiating into hepatocyte or cholangiocyte-like cells implicated CD24a as a possible marker for hepatic progenitors (Ochsner et al. 2007). None of the aforementioned markers are specific to oval cells, making the isolation and study of oval cells difficult. Nonetheless, it has been suggested that upon injury, these cells contribute to liver regeneration (reviewed by Alison et al. 1996, Walkup and Gerber 2006). Following toxic depletion of mature hepatocytes, oval cell proliferation was shown to cause liver regeneration. The study of tumor necrosis factor (TNF) family members as well as lymphotoxin  $\alpha$ , that modulate the liver mass, indicated that both mature hepatocytes and oval cells respond to these cytokines. It is therefore unclear what the relative role of these precursors in liver mass maintenance is (reviewed by Fausto 2005).

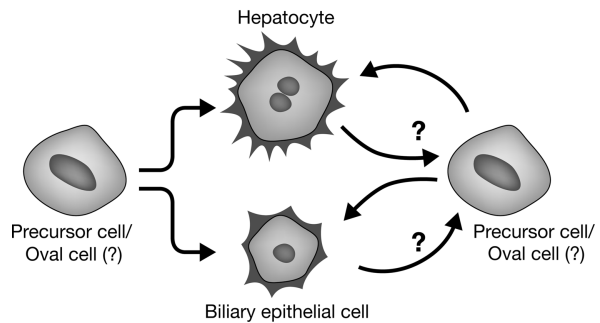
It has been questioned whether oval cells are the only existing liver precursor cells. Cells bearing markers such as CD29, CD73, CD44, and CD90, that lack hemopoietic cell markers (including Thy-1) and lack cytokeratin-19, both being oval cell makers, were found to differentiate into mature hepatocytes in culture (Herrera et al. 2006). Surface expression of EpCAM was used as a means to purify cells directly from the liver (Schmelzer et al. 2007). These cells gave rise to hepatocytes both in vitro, and upon in vivo transplantation. Analysis of cell populations isolated by use of EpCAM and Thy-1 markers showed that more than one cell type is found in the selected population. One of the cell types possessed oval cell markers and, unexpectedly, mesenchymal markers (Yovchev et al. 2008). This complex gene expression pattern could mean that epithelial/mesenchymal transitions (EMTs) (reviewed by Prindull and Zipori 2004) occur in the liver. Other implicated populations of liver progenitors are the hepatic SP cells that lack hemopoietic markers (Tsuchiya et al. 2007).

In addition to the putative resident stem cells, liver recovery from damage has been suggested to result from recruitment of bone marrow cells. These cells seem to contribute to hepatocyte formation, either through transdifferentiation (Jang et al. 2004) or through fusion (Camargo et al. 2004) (see Chapter 6).

Although several studies suggest the existence of candidate precursor liver cells, most of these studies are related to fetal development, whilst the rest relate to

injury. The study of liver precursors at steady state has not yielded conclusive information. It should be taken into account that in the adult, mature liver cells themselves are responsible for most of the liver steady-state regeneration (Michalopoulos and DeFrances 1997). Whether during injury other cells (oval cells?), perform the massive regeneration, remains to be conclusively determined. It is remarkable that the study of the liver, an organ that shows regenerative capacity comparable to that of the hemopoietic system, and is similar, in its versatility, to organs of amphibians, has not yielded a clear demonstration of tissue-specific stem cells. It should therefore be considered that the liver's outstanding regenerative ability is due to the capacity of hepatic cell populations to undergo transitions from a mature cell phenotype into a stem-like state, enabling their regenerative activity (Fig. 2.1). This is reminiscent of the plant cell system, which presents reversibility between stemness and cell maturity. Similarly, the regeneration of limbs in amphibians proceeds through processes of transdifferentiation and dedifferentiation (see Chapter 6). There is a good reason to believe that these processes have been preserved during evolution and exist, to an extent, in mammalian tissues such as the liver. The existence of the hepatic cell hierarchy, in which stem cells give rise to progenitors than ultimately mature cells, is often implied (Zhang et al. 2008). However, the available data are also compatible with a non-hierarchical model, in which transitions among cell types occur and the search for hepatic stem cells is ongoing (Walkup and Gerber 2006).

**Fig. 2.1** Precursor cells in the liver (*left*) are shown to give rise to two cell types, hepatocytes and biliary epithelial cells (*middle*). This process could, however, be reversible (*right*): The apparent ability of hepatocytes to proliferate extensively might be due to transitions between the mature and the precursor state

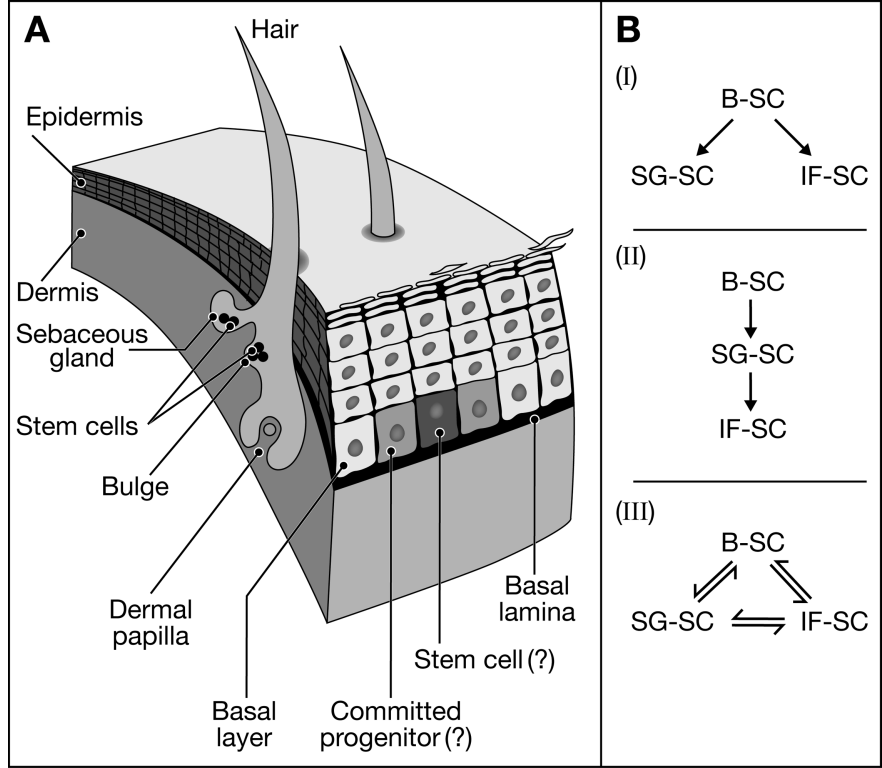


## 2.2 Tissue-Specific Stem Cells from Organs Undergoing Extensive Repopulation and Repair

### 2.2.1 Skin Stem Cells

The skin epidermis is a multilayered epithelium of keratinocytes (Fig. 2.2A). A thick ECM (basal lamina) separates the epidermis and the deeper skin part, the dermis. A basal layer of relatively undifferentiated keratinocytes is attached to the basal lamina. This layer contains a large number of proliferating cells. The epidermis is

organized into columns of cells, starting from the basal layer. The division of cells in the basal layer forces a fraction to move outwards and towards the skin surface, thereby building the next layers. As the cells move outwards, they differentiate until they become squame. At this terminal stage of differentiation, the cells detach from the skin surface and are shed. The hair follicle constitutes a distinct structure within the skin. The outer surface of the hair follicle (outer root sheath) is an extension of the basal layer of the epidermis. It engulfs an inner part of the hair made of three concentric cell layers (inner root sheath). The deepest part of the follicle, called the matrix, surrounds a cluster of mesenchymal cells, the dermal papilla. Close to the skin surface below the sebaceous gland, a hair structure called the bulge contains a cluster of cells situated just beneath the outer root sheath.



**Fig. 2.2** Skin structure and skin progenitors: **(A)** Skin cellular organization, and **(B)** hypothetical relationships among skin progenitors; **(I)** the bulge stem cells give rise to other stem cells, **(II)** a putative hierarchy topped by the bulge stem cell, and **(III)** transitions between stem cell types. B-SC = bulge stem cells, IF-SC = interfollicular stem cells, SG-SC = sebaceous gland stem cells

Cells in the basal layer of the epidermis were assumed to be the stem cells responsible for the extensive and constant new cell production occurring throughout mammalian life. This assumption has gained support from cell culture experiments.

Epidermal cells proliferate extensively either in long-term cultures, in the presence of supportive fibroblasts, or when supplied with growth factors such as epidermal growth factor (EGF) (Rheinwald and Green 1977). The most extensive growth is obtained by culturing cells from the basal layer, as compared to the other layers of the epidermis (reviewed by Alonso and Fuchs 2003). The basal layer was therefore first supposed to be the site of epidermal stem cell residence. Not all cells in the basal layer were equal in their proliferation *in vitro*, indicating that only a fraction of these cells may be stem cells. It is thought that the first step of differentiation of basal layer stem cells is the production of transiently amplifying progenitor cells, that further differentiate to form the epidermal layer of differentiating cells (Fig. 2.2A).

To further identify putative skin stem cells, a pulse-chase approach using nucleotide analogs was used. Proliferating cells incorporate bromodeoxyuridine or tritiated thymidine. Upon chase, the label disappears from rapidly proliferating cells, and is retained by cells that divide at a slow rate. Experiments in which labeling with nucleotide analogues was performed, showed that most of the label-retaining cells are found within the hair follicle, and in the bulge area in particular, rather than in the interfollicular basal layer that was the first suspected niche for epidermal stem cells (Fig. 2.2A). These findings correlated well with the observation that cells endowed with a potential for extensive proliferation *in vitro*, are mostly found in the bulge (Blanpain et al. 2004, Cotsarelis et al. 1990, Taylor et al. 2000, Tumber et al. 2004), while only a small fraction can be extracted from the skin basal layer or the interfollicular space. Studies that followed, asserted that upon wound healing, both stem cells in the epidermal basal layer and in the bulge, contributed to skin regeneration. However, only progeny of the former contributed to long-term skin regeneration, whereas the progeny of the bulge cells did not contribute to long-term skin maintenance (Ito et al. 2005). A recent study proposed that both cell sources do, indeed, contribute to wound healing in a long-term manner (Levy et al. 2007). In addition to the above, cells at the base of the sebaceous gland were also suggested as candidate epidermal stem cells.

Cells with stem cell properties were thus identified in the basal layer of the epidermis within the interfollicular spaces, in the bulge, as well as in the sebaceous gland. It remains to be determined whether these cells are arranged in a hierarchy, or whether they are site-specific (Fig. 2.2B), with each of them contributing to a specific part of the epidermis. It is also unclear whether there are inter-conversions among the different epidermal stem cell populations, and whether cells that do not have stem cell functions may acquire such a phenotype upon stress. Indeed, the possible transition of interfollicular cells into hair follicle stem cells has been suggested. The highest proliferation potential, and dye retention, is ascribed to bulge stem cells. Since these are multipotent, and apparently the only epidermal cells that maintain multipotency in adulthood, it has been suggested that they give rise to the other epidermal stem cells. One study utilized the K-15 promoter with an inducible Cre recombinase, as well as enhanced (E) GFP, to label bulge stem cells. It was found that bulge cells generated all epithelial cells within the follicle and hair. In addition, some labeled cells were found in the sebaceous gland and in the interfollicular spaces, although at a lower incidence (Morris et al. 2004). Several studies suggested

that bulge stem cells do not give rise to interfollicular stem cells (Claudinot et al. 2005, Ito et al. 2005, Levy et al. 2005). This situation, which presents uncertainty concerning the existence of a single cell type being the precursor of all epidermal cells, is very reminiscent of the status of hemopoiesis in the 1960s, prior to the identification of the HSC (see Chapter 1). Indeed, a recent study challenged the notion of the bulge being the origin of hair follicle stem cells, and suggested that Lgr-5 positive cells in the outer root sheath of the cycling portion of the follicle, constitutes the hair follicle progenitor population (Jaks et al. 2008, reviewed by Morgan 2008).

As is the case for the HSCs, the skin itself, as well as isolated and cultured skin cells, can be transplanted back into animals and humans and contribute to new skin formation. Hair follicles were a source for long-term in vitro passaged populations that could be then implanted back and participate in follicle formation in vivo (Claudinot et al. 2005). There seems to be great promise in the ease of skin cell propagation in culture. However, the clinical practice is based, to date, on skin-fragment transplantation, rather than on the use of cultured cells.

To date, there is no clear demonstration of epidermal stem cell markers. It is possible that such specific markers do not exist. This is mainly due to the fact that epidermal stem cells reside in many skin niches, and seem to be heterogeneous in nature. Moreover, it is impossible to discriminate between the “skin stem cells,” and their transiently amplifying progeny. The basic property of epidermis cells, regarded as a stem cell property, is the capacity to extensively proliferate in vitro in a long-term fashion. This is regarded as demonstrating the self-renewal capacity of the epidermal stem cells. Obviously this definition of self-renewal is rather different from that based on the study of HSCs. The latter self-renew only when cultured with bone marrow stroma, and to a very limited extent. Even under in vivo repeated passages HSCs decline and fail to “continuously grow” (this issue is further discussed in Chapter 5).

Attempts were made to identify epidermal stem cell molecules, characteristic of this stem cell type. Epidermal cells bearing relatively high  $\alpha 2$  integrin possess a relatively higher proliferation potential. Additional integrin family members,  $\alpha 6$  and  $\beta 1$ , are considered to be possible skin stem cell markers. These molecules mediate the adhesion of epidermal stem cells to the basal lamina. It is, however, obvious that these molecules are also shared by other skin cells and, in any case, are not skin-specific. As would be expected, keratins are also common to all skin stem cells. Indeed, keratin 15 is suggested as a bulge cell maker. In addition, the cell surface marker CD34, often used to purify HSCs, was detected in skin cells. However, HSCs negative for this marker have equally been found to possess long-term hemopoietic repopulating abilities. Therefore, this molecule should not be regarded as a common marker for HSCs or skin stem cells. Apart from integrins and cyto-keratins, several signaling cascades have been identified in epidermal stem cells. The Wnt pathway is involved in bulge cell differentiation into follicular cells. An abundance of Wnt signaling inhibitors is found in early epidermal stem cells, probably in order to prevent differentiation and preserve the stem cell pool. Other pathways implicated are TGF $\beta$  and fibroblast growth factor (FGF)-1 signaling (Khavari 2004).



A less studied population within the hair follicle is the melanocyte progenitor pool. The latter has been suggested to exist within the lower, permanent portion of the hair follicle. However, the relationship of the presumptive melanocyte stem cells and other stem cell populations is unclear. Melanocyte precursors originate, during embryogenesis, from the neural crest, similar to an additional hair stem cell population, the skin progenitor cells (SKPs). The latter are found in the hair papilla, are mesenchymal in nature, and exhibit a pluripotent nature (see Chapter 3).

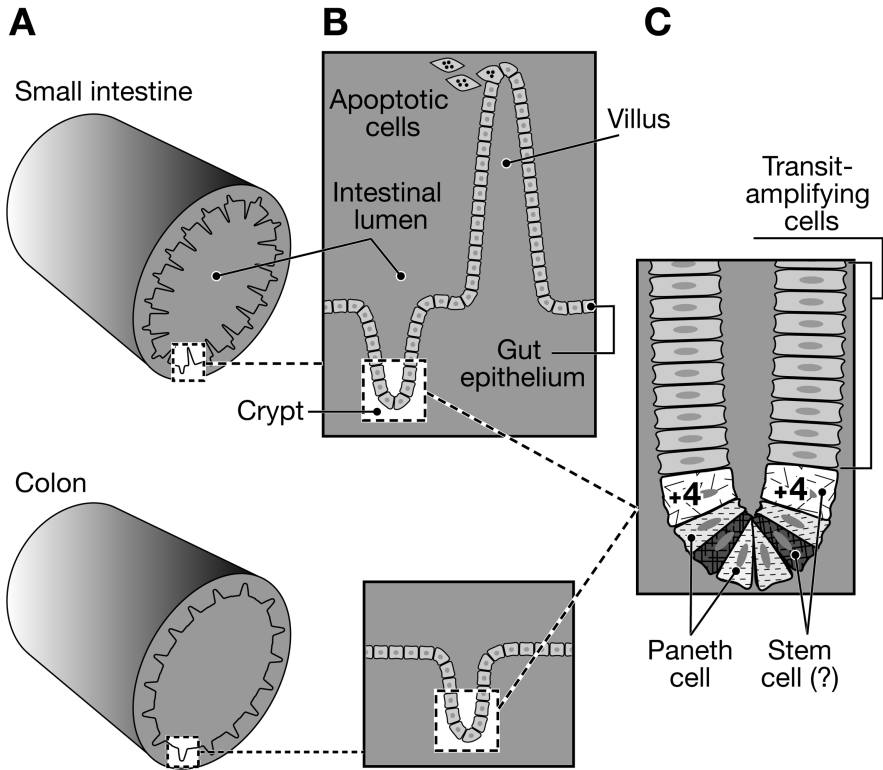
### ***2.2.2 Gut Stem Cells***

The intestinal wall is a direct ectodermal continuation of the outer skin, and is similarly constantly exposed to the external environment, through food and other material consumption. It is endowed with a capacity, which exceeds that of the skin, to repopulate extensively. The small intestine wall surface sends out protrusions called villi into the intestinal lumen. The colon surface does not harbor such protrusions (Fig. 2.3A). In both the intestine and the colon, the intestinal wall has invaginations of epithelium, called crypts. In the case of the small intestine, these are placed at the base of the villus (Fig. 2.3B). The crypt has been suggested to contain gut stem cells (Winton and Ponder 1990). The very base of the crypt is made of Paneth cells, which are one type of mature intestinal cell (Fig. 2.3C). Adjacent to these, cells at position +4 in the crypt have been suggested as candidate small intestine stem cells (Potten et al. 1997). This possibility is supported by the long-term retention of DNA labeling exhibited by +4 cells. The intestinal stem cells give rise to transit-amplifying cells, which are committed to differentiation and give rise to mature Goblet cells, enteroendocrine cells, and absorptive cells. These three mature cell types are secretory cells. The intestinal stem cells are multipotent, and give rise to all the mature cells found in this organ (Cheng and Leblond 1974). As the cells divide and push their way up the villus, they mature until reaching the villus tip, where they undergo apoptosis and are shed into the gut lumen.

The positioning of gut precursor cells in the crypt is certain. However, the identity of the stem cells has been recently challenged by a study that identified a different candidate stem cell. The G protein-coupled receptor, *Lgr5* is a Wnt target gene. The expression of this gene is restricted, in the intestinal epithelium, to columnar epithelial cells at the base of the crypts. Fate mapping with a Cre knock-in allele of *Lgr5* showed that individual *Lgr5* crypt base columnar (CBC) cells self-renew in vivo and give rise to all intestinal epithelial lineages (Barker et al. 2007). These cells were identified both in the small intestine and in the colon. Contrary to the +4 position cells, that seem to be quiescent, the CBC cell is dividing, with a cycle time of about one day. This cell is less sensitive to irradiation, compared with +4 cells.

By analogy to HSCs, the committed amplifying cell population of the gut may be expected to differentiate terminally in a unidirectional manner, while losing the capacity to proliferate. Nevertheless, it has been suggested that these cells may revert back to stem cells (Potten 1998, and reviewed by Crosnier et al. 2006). It is intriguing to note that during development, the villus forms prior to the emergence of the crypt. Thus, villus cells may precede, and give rise to, stem cells of the crypt.





**Fig. 2.3** Gut tissue structure and gut progenitors: the small intestine and colon differ in surface structure: (A) The former has protrusions (villi) and invaginations (crypts), whereas the latter has crypts only. The cellular composition of these structures is shown in (B) and details of the stem cell niche within the crypt are shown in (C)

In fact, crypts and their associated stem cells appear in the intestine only several weeks after birth. The apparent “reversed” development of stem cells of the crypt seems, therefore, to be in line with the assumption that transit-amplifying progenitor populations may revert into stemness. One further observation is that commitment to a secretory pathway does not abolish proliferation capacity. Thus, extensive proliferation in the intestine is not restricted to the transit-amplifying cell populations and is shared, as in the case of the liver, with some mature cells.

### 2.3 Tissue-Specific Stem Cells in Tissues and Organs Exhibiting Moderate Repopulation and Repair Capabilities

It is often asserted that skeletal muscles have a remarkable regenerative capacity. This is evident when one considers situations in which extensive exercise results in the increase of muscle volume, or following toxic damage that sporadically kills cells within muscles. However, contrary to the hemopoietic system, that can be

depleted to almost zero and nevertheless regenerates, partial amputation of a muscle would not be followed by re-formation of the missing part. The skeletal muscle is therefore classified herein as a tissue with a moderate regeneration capacity.

### ***2.3.1 Skeletal Muscle Stem Cells***

Skeletal muscle is made of myofibers, the contractile units which are giant syncytial cells containing hundreds of nuclei. Skeletal muscles show considerable repair capabilities upon toxic damage that reduces the number of viable and functional myofibers, but leaves the overall muscle structure intact. Myofibers are terminally differentiated cells, and therefore are not likely to participate in muscle regeneration following injury. However, the muscle contains an additional cell type, the satellite cell. These are mononuclear cells, positioned between the plasma membrane of the muscle fiber, and the ECM-basal lamina that wraps the muscle fiber (Mauro 1961, also reviewed by Collins et al. 2005). Myofibers can be isolated and cultured, and under such conditions, it was possible to show that satellite cells contribute to the regeneration of the myofiber (Bischoff 1975, Konigsberg et al. 1975). However, upon their separation from the myofiber and isolation to homogeneity, satellite cells do not exhibit any significant repair capabilities. It was therefore anticipated that satellite cells are progeny of an earlier precursor cell, either within the muscle (Asakura et al. 2002), or from an external source, such as the bone marrow (LaBarge and Blau 2002). Nevertheless, studies with intact myofibers demonstrate the high repair capacity of satellite cells. The discrimination between myofibers and satellite cells in these experiments was based on the use of transgenic mice in which the  $\beta$ -galactosidase gene is under the promoter of the myosin light chain 3F gene. The resulting expression is confined to myofiber nuclei, and not to the satellite cells. The latter are identified by the expression of the Pax-7 gene. Single myofibers were individually transplanted in vivo into the muscles, in a mouse inflicted with muscle dystrophy due to a deficiency in the dystrophin gene. The host muscle was irradiated, prior to transplantation, to prevent the participation of host satellite cells in the regeneration process. Under these conditions, an individual myofiber harboring as few as seven satellite cells yielded, in a matter of 3 weeks, 1,000 new myofibers with thousands of nuclei (Collins et al. 2005). Ultimately, it was demonstrated that a single muscle progenitor cell, transplanted into mouse muscle, proliferates and differentiates, contributing to the muscle fiber (Sacco et al. 2008). The satellite cell is therefore strictly dependent upon its niche, and while within this niche, under demand conditions, it is capable of extensive generation of muscle myofibers.

One open question is whether the satellite cell is the earliest muscle stem cell, or whether it is preceded by yet a more primitive cell. An additional issue is the possible existence of a hierarchy downstream of the satellite cells; Pax-7<sup>+</sup>Myf<sup>-</sup> satellite cells give rise to Pax-7<sup>-</sup>Myf<sup>+</sup> cells that do not replicate, and undergo terminal differentiation (Kuang et al. 2007 and reviewed by Zammit 2008).

Following muscle injury, damaged myofibers retain the basal lamina and the associated satellite cells. This enables the subsequent repair, in which satellite cells not only participate in regeneration of their host myofiber, but also migrate to neighboring myofibers. This phenomenon raises two questions that should be addressed by future research: Firstly, why and how are satellite cells spared, following muscle damage? Secondly, since these cells are endowed with a migratory capability, why is it so difficult to isolate them in an intact form, capable of repopulation?

### ***2.3.2 Cardiovascular Progenitor Cells***

Similar studies have been conducted with heart muscle. It was found that cells with a phenotype of Lin<sup>-</sup>cKit<sup>+</sup> (CD34<sup>-</sup>), can be isolated from Fischer rats and cultured for over a year without losing their proliferation potential. These cells were shown to be multipotent at the clonal level, i.e. single cells gave rise to cardiomyocytes, vascular smooth muscle cells (VSMCs), and endothelial cells. Upon transplantation into infarcted rat heart, the cells differentiated into the above progeny (Beltrami et al. 2003). Similarly, cKit<sup>+</sup> cells were demonstrated in adult human heart (Bearzi et al. 2007). Indeed, studies on heart development indicated that embryonic cardiac progenitor cells are tri-potent. In addition to the Lin<sup>-</sup>cKit<sup>+</sup> population, cells that are Sca-1 positive, and SP cells that are Hoechst excluding, have also been reported to have similar progenitor properties (Martin et al. 2004, Oh et al. 2003). Whether all these populations are related, arranged in some hierarchy, or whether they all represent genuine cardiac progenitors, remains to be determined.

## **2.4 Tissue-Specific Stem Cells in Tissues and Organs Exhibiting Poor Regeneration and Repair Capabilities**

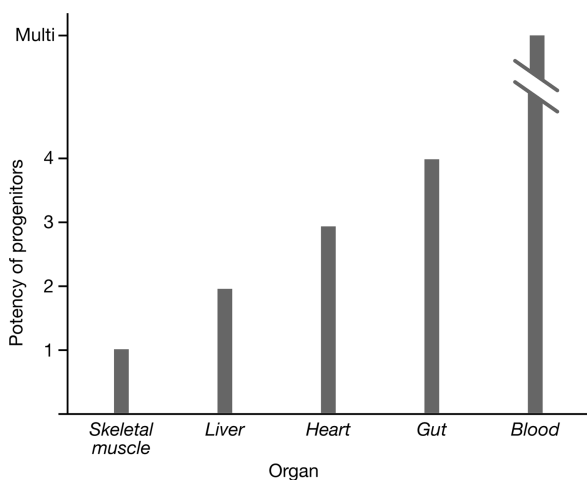
The idea that only regenerating tissues contain stem cells was particularly logical, in view of the ineffective correction of damage inflicted either on the central or the peripheral nervous systems. These tissues have consequently been thought to be devoid of stem cells. The identification of cell proliferation, within the adult brain came, therefore, as a surprise to many.

Cell replacement by activity of neural stem cells (NSCs) may occur in mammals, even though the capacity of the mammalian brain to regenerate is low. NSCs were identified following the culture of cells isolated from the nervous system (Chiasson et al. 1999, Doetsch et al. 1999, Johansson et al. 1999, Rietze et al. 2001, Stemple and Anderson 1992, and reviewed by Temple 2001). A potent manner to culture such NSCs was the creation of neurospheres (Nunes et al. 2003). Progenitors isolated from adult human white matter, marked by GFP under an early oligodendrocyte promoter, CNP, were seeded in medium supplemented with the cytokines bFGF, neurotrophin3 (NT3), and platelet-derived growth factor (PDGF)-AA. Upon transfer to medium with bFGF only,

neurospheres were generated that differentiated into neurons and glia both in vitro and in vivo, as verified by the GFP labeling. NSCs have been suggested to harbor surface CD133 antigen. A recent study (Coskun et al. 2008) showed that NSCs were CD133<sup>+</sup>CD24<sup>-</sup> whereas the rest of the cells in rodent brain either lacked CD133 or were CD133<sup>+</sup>CD24<sup>+</sup>. This, however, is not a prerequisite, and cells that have NSC properties but lack CD133 have also been identified. The issue of markers is discussed in detail with regard to HSC (Chapter 1), in order to highlight the fact that cell surface markers are not a sufficient determinant of stemness. NSCs have been identified in many sites in the nervous system, as shown by their isolation from these sites and subsequent in vitro growth and differentiation. The stem cell potential of NSCs is best demonstrated by their transplantation, in vivo, into the brain. However, an effective method for the analysis of NSCs, at the single cell level in vivo, is unavailable.

## 2.5 Conclusions, Questions, and Enigmas

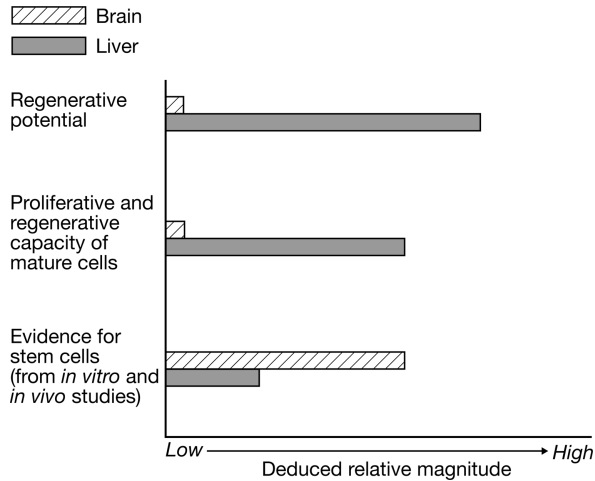
Are tissue-specific stem cells similar to HSCs, and do they represent a homogeneous group? This is clearly not the case: Fig. 2.4 shows that marked differences exist in the potency of the different populations, ranging from multipotency of the HSC, through oligopotency of cells in the ocular surface (Majo et al. 2008), to unipotency of cells such as muscle progenitors. Tissue-specific stem cells from different organs also differ dramatically with regard to their repopulating or regenerative capacities. Those of the nervous tissue would be placed at the bottom of the potency list, whereas those of the skin would be placed high on such a list. Is this due to the fact that each tissue-specific precursor population has a different, built-in capacity for repopulation? Alternatively, various tissues may impose different degrees of restric-



**Fig. 2.4** Tissue-specific stem cells are highly divergent in their differentiation potency, as assessed by the number of different progeny they are capable of giving rise to

tions on stem cell activity. In the absence of definitive data to support either of these alternatives, the major differences in potencies among tissue-specific stem cells, remain unexplained. There are other clear differences among tissue-specific progenitors. Muscle satellite cells hardly proliferate *in vitro*, whereas epidermal progenitor cells can be propagated extensively and for prolonged time periods in culture. This last property is not shared by HSCs that are, in this respect, closer to satellite cells. Similar major differences exist in terms of transplantation capacity. Of major importance, for future research, is the poor evidence for tissue-specific stem cells in the liver and the possibility that adult epithelial hepatic cells can proliferate extensively and account, at least partially, for regeneration of this organ (Fig. 2.5).

**Fig. 2.5** A discrepancy between the cellular content of an organ and the regenerative potential it possesses: The liver has a high regeneration potential, but has not been shown to unequivocally contain potent stem cells. By contrast, the brain has poor regeneration potential, but highly proliferating NSCs have been extracted from this organ and propagated in culture



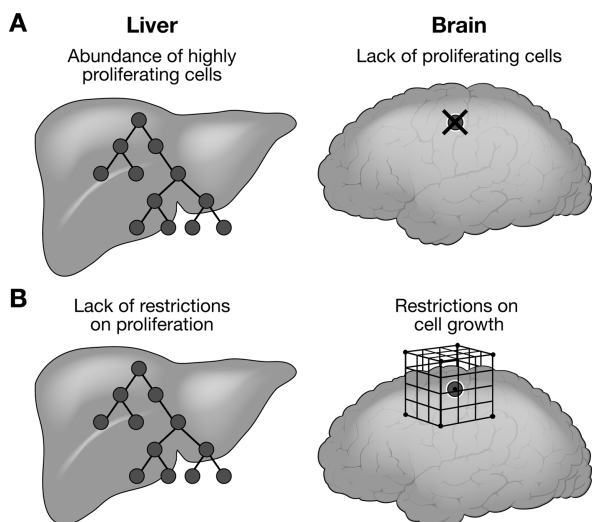
The common denominator of tissue-specific stem cells is, therefore, neither proliferation potential or repopulation capacity, nor is, it their ability to migrate. The commonality boils down to one single property, i.e. being precursors of the mature cells in the organ. This is a purely descriptive definition, which may have no common molecular basis (Box 2.2). Indeed, by the same token, a pre-T cell, is the precursor of the T cell, and the resting macrophage is the precursor of the activated macrophage. Should one then designate the pre-T cell as a stem cell of the antigen-specific T cell? In Chapter 6, stem cells are redefined and a solution to this dilemma is suggested. Here, it suffices to say that a group of cells, which are “precursors of a next step in differentiation,” do not constitute a homogenous biological entity. For example, fertilization and parthenogenesis, or artificial nuclear transfer, may all lead to the formation of an embryo. However, it is inconceivable to classify sexual reproduction, parthenogenesis and artificial nuclear transfer as one biological phenomenon, since these processes are completely divergent, at the molecular level, despite the fact that all these terminate in formation of an embryo. By the same token, steps in the maturation of T cells are molecularly divergent from the

transition between an HSC to a committed progenitor, and the classification of both as “stem cell events,” has no molecular sense and is thus misleading.

### Box 2.2 The definition of stemness-Stage II

The HSC is but one multipotent tissue-specific stem cell. Several tissues and organs contain similar stem cells residing in specific niches. Some, but not all, of these are quiescent, and have migratory and transplantation capacities. Other alleged tissue-specific stem cells, have an oligopotent or a unipotent phenotype, and share only one common property with the others, i.e. being the precursors of mature cells of their organ of residence.

The liver, much like the bone marrow, has a remarkable regenerative potential. This is partially due to the capacity of mature hepatocytes, to contribute by proliferation to liver regeneration. Specific mature cells of the gut may share this property. The mature pancreatic  $\beta$  cells may similarly be capable of proliferation (Dor et al. 2004). What then, is the specific trait of hepatocytes that endows them with this outstanding proliferation capacity? Why don't most other organs possess such potent mature cells? One possible explanation for this phenomenon is the assumption raised above, that hepatocytes are more versatile than other cells and are able to perform transitions into a stem cell state. This may explain why cells sharing properties of HSCs, hepatocytes and mesenchymal cells are found in the liver. Should such transitions between the differentiated state and stemness occur frequently in the liver, then many types of intermediate cell types will be present at any given time point. This may explain some of the variability, and lack of consensus, as to the nature of liver precursors. Future studies should be designed



**Fig. 2.6** The lack of overt growth of progenitors in particular organs could either be due to lack of proliferation potential (A) or to restriction of growth, imposed by the organ microenvironment (B)

to examine the properties of adult hepatocytes compared to other mature cell types, in order to determine the molecular basis of hepatocyte versatility. Do mature hepatocytes maintain operative signaling cascades that characterize oval cells? Are hepatocytes capable of dedifferentiation? An alternative explanation for the differences between the liver and other organs, is that the liver lacks restrictive signals that limit plasticity of cell behavior. According to this assumption, one should identify the restrictive machinery in organs such as brain, which is presumably missing in the liver (Fig. 2.6).

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